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Interbacterial competition assay

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Updated date: May 10, 2023

An abbreviated version of this protocol was published in eLIFE in Oct 2022

Antibacterial T6SS effectors with a VRR-Nuc domain are structure-specific nucleases

DOI: 10.7554/eLife.82437

Detailed protocol

Interbacterial competition assay

Day 1

Bacterial strains of *Salmonella bongori* (See Supplementary file 7), and *Escherichia coli* K-12 W3110 pEXT22 Km^R were streaked in Lysogeny Broth- agar plates (10 g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract, 1,5% agar) – 90mm plates with 25 mL of medium with additional antibiotics as needed. All strains were grown for 12-16 h (overnight, ON) under 200 rpm at 37 ºC.

Day 2

Single colonies of bacterial strains were inoculated in 3 mL of LB with appropriate antibiotics in 15 mL tubes and grown ON at 37 °C under 200 rpm.

Day 3

ON cultures of *S. bongori* (WT, Δ*tssB*, Δ*tssB* pFPV25.1*tssB* Amp^R, Δ*vgrG1*, Δ*vgrG2*, or Δ*vgrG3*) as attackers and *E. coli* K-12 W3110 pEXT22 Km^R or *S. bongori* (Δ*tseV2/tsiV2.1/tsiV2.2* Km^R, Δ*tseV2/tsiV2.1/tsiV2.2* Km^R pFPV25.1*tsiV2.1*, Δ*tseV3/tsiV3* Km^R or Δ*tseV3/tsiV3* Km^R pFPV25.1*tsiV3*) as prey were subculture in 5 mL of LB (1:30 dilution) in a 50 mL tube. Cultures were grown at 37 ºC under 200 rpm until reaching $OD_{600\ nm}$ 1.6, then adjusted to $OD_{600\ nm}$ 0.4 and mixed with vortex in a 10:1 ratio (attacker:prey) in a 1,5 mL tube (200 µl:20 µl).

Input preparation –5 µL of the mixture were transfer to a 1.5 mL tube with 995 µL of LB medium and homogenized by vortex. Serial dilutions were prepared by transferring 100 µL to 900 µL of LB up to 10^3 and plated on LB agar plates with antibiotics for prey selection. Plates for input were grown ON at 37 ºC. CFU (colony-forming units) were recorded.

Output preparation - 1x1 cm nitrocellulose membranes (pore 0.22 µm) were place on a sterile 90 mm petri dish and 5 µL of the mixture were spotted onto the membrane. After the mixture was absorbed by the membranes, these were immediately transferred and positioned face-up in a 90 mm plates with 40 mL of LB-agar - carefully to avoid bubble formation between medium and membrane. The membranes for output were incubated at 37°C with the membrane facing up. After the estimated time of competition, the membranes were transfer from the plates to a 1.5 mL tube with 1 mL of LB medium and homogenized by vortex. Serial dilutions were prepared up to 10^5 and plated on 25 mL LB agar plates with antibiotics for prey selection. Plates for output were grown overnight at 37 ºC. CFU counts were recorded.

The prey recovery rate was calculated by dividing the CFUs counts of the output by the CFU of the input. The rates were normalize by the values recovered for *S. bongori* WT as control. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. **$p < 0.01$, and ***$p < 0.001$.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Bayer-Santos, E. and Sanchez-Limache, D. E.(2023). Interbacterial competition assay. Bio-protocol Preprint. bio-protocol.org/prep2287.
2. Hespanhol, J. T., Sanchez-Limache, D. E., Nicastro, G. G., Mead, L., Llontop, E. E., Chagas-Santos, G., Farah, C. S., de Souza, R. F., Galhardo, R. D. S., Lovering, A. L. and Bayer-Santos, E.(2022). Antibacterial T6SS effectors with a VRR-Nuc domain are structure-specific nucleases. eLIFE. DOI: 10.7554/eLife.82437